MODEL OF RFP PRODUCTION

1. Modelling

We want to characterize the promoter's velocity of expression due to pressence of mercury, so we will attach an RFP gene to it to produce.

First we will model the production of RFP due to Hg^{2+} at Estacionaria time phase with this configuration. Then we add the initial phase consideration.

1.1. Estacionaria time phase.

The GMO we have is

- J23100 Promoter + MerR + Terminator + Promoter nP + RFP + Terminator

Where MerR is a represor and releases when H_g^{2+} is presented in the interior of the cell, we will note this amount as $H_{g_{in}}$ and the exterior as $H_{g_{out}}$. And Promoter nP is the promoter which velocity we want to characterize.

The activation of the gene in the pressence of $H_{g_{in}}$ is represented like:

- $G + H_{g_{in}}^{2+} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} X$

where G is the inactive form of gene repressed by merR, and X is its active form.

Through the law of mass action we derive the diferential equation

$$\frac{d[X]}{dt} = k_1[G][H_{g_{in}}^{2+}] - k_{-1}[X] \tag{1}$$

At the equillibrium state,ie. $\frac{d[X]}{dt} = 0$, we have that the proportion of genes in the activated gene is

$$\frac{[X]}{[X]+[G]} = \frac{[H_{g_{in}}^{2+}]}{K_D + [H_{g_{in}}^{2+}]} \tag{2}$$

where $K_D = k_{-1}/k_1$.

So this is the average production rate of a typical gene, so the average mRNA production will be

$$\frac{d[mRNA]}{dt} = \alpha + k_1 \frac{[H_{g_{in}}^{2+}]}{[H_{g_{in}}^{2+}] + K_D} - \delta_1[mRNA] \tag{3}$$

Where α is the production of mRNA due to the stochastic nature of the binding of merR for repressing the RNA Polymerasa and δ_1 is the degradation factor of mRNA.

The reaction for production of RFP from mRNA is

- $mRNA \xrightarrow{k_2} RFP \xrightarrow{\delta_2}$

so we derive the diferential equation

$$\frac{d[RFP]}{dt} = k_2[mRNA] - \delta_2[RFP] \tag{4}$$

where δ_2 is the degradation constant of $[RFP]$.

The variation of exterior mercury is

- $H_{g_{out}}^{2+} \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} H_{g_{in}}^{2+}$

from here we derive the differential equation

$$\frac{d[H^{2+}_{g_{out}}]}{dt} = k_{-3}[H^{2+}_{g_{in}}] - k_3[H^{2+}_{g_{out}}] \tag{5}$$

As the interior mercury is used by the inactive form of the gene, the variation of $H^{2+}_{g_{in}}$ is

$$\frac{d[H^{2+}_{g_{in}}]}{dt} = k_3[H^{2+}_{g_{out}}] - k_{-3}[H^{2+}_{g_{in}}] - k_1\frac{[H^{2+}_{g_{in}}]}{K_D + [H^{2+}_{g_{in}}]} \tag{6}$$

To solve this system we will assume that the permeability of the cell memebrane to mercury is instantaneus, because mercury pass very easy by it, then we have that $\frac{d[H^{2+}_{g_{out}}]}{dt} = 0$ then $k_{-3}[H^{2+}_{g_{in}}] = k_3[H^{2+}_{g_{out}}]$ so we have that the variation of the interior mercury is

$$\frac{d[H^{2+}_{g_{in}}]}{dt} = -k_1\frac{[H^{2+}_{g_{in}}]}{K_D + [H^{2+}_{g_{in}}]} \tag{7}$$

And assume that mRNA is at Quasi-Steady state, ie. $\frac{d[mRNA]}{dt} = 0$, then we have that the amount of mRNA is

$$[mRNA] = \frac{\alpha}{\delta_1} + \frac{k_1}{\delta_1}\frac{[H^{2+}_{g_{in}}]}{[H^{2+}_{g_{in}}] + K_D} \tag{8}$$

Then we have that the velocity of production of RFP is

$$\frac{d[RFP]}{dt} = k_2(\frac{\alpha}{\delta_1} + \frac{k_1}{\delta_1}\frac{[H^{2+}_{g_{in}}]}{[H^{2+}_{g_{in}}] + K_D}) - \delta_2[RFP] \tag{9}$$

So we will also assume that the degradation of RFP is very slow, $\delta_2 = 0$, and also the stochastic production of mRNA is very slow, $\alpha = 0$. Then finaly we have that the production of RFP is

$$\frac{d[RFP]}{dt} = V_{max}\frac{[H^{2+}_{g_{in}}]}{[H^{2+}_{g_{in}}] + K_D} \tag{10}$$

where $V_{max} = k_2\frac{k_1}{\delta_1}$

1.2. Initial phase. Since bacteria grow exponentially, it is often useful to plot the logarithm of the relative population size $[Y = ln(N/N_0)]$ against time .So lets use the Gompertz equation to model this. The three phases of the growth curve can be described by three parameters: the maximum specific growth rate, μ_m is defined as the tangent in the inflection point; the lag time, λ, is defined as the x-axis intercept of this tangent; and the asymptote $[A = ln(N/N_0)]$ is the maximal value reached. Here we are not considering the death rate. [1]

$$Y(t) = Ae^{-e^{\frac{\mu_m e}{A}(\lambda - t)+1}} \tag{11}$$

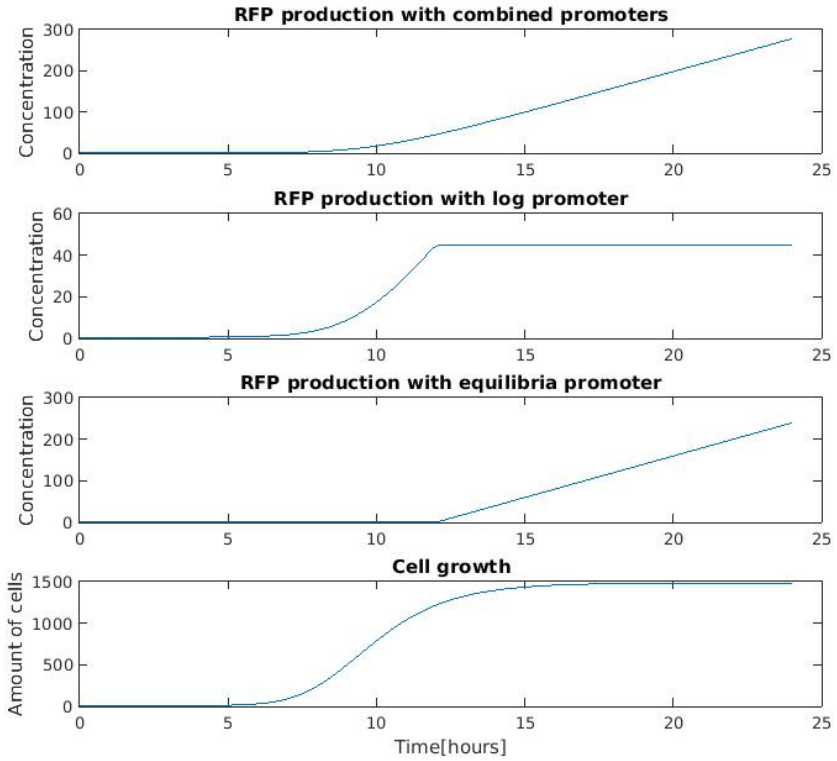
Lets add that the amount of bacteria changes to our model. So we have more bacteria. As $Vmax$ depends on the amount of bacteria, so we can propose that a single bacteria has a velocity $V'_{max} = \frac{V_{max}}{N_{max}}$ production of RFP per unit of time and then

$$Vmax(t) = N(t)V'_{max} = V_{max}\frac{N(t)}{N_{max}} = V_{max}\frac{e^{Y(t)}N_0}{N_{max}} \tag{12}$$

So our model now is

$$\frac{d[RFP]}{dt} = e^{Y(t)}N_0\frac{V_{max}}{N_{max}}\frac{[H^{2+}_{g_{in}}]}{K_D + [H^{2+}_{g_{in}}]} \tag{13}$$

Here we are assuming that when a bacter appears it alredy has all the properties necessary to work, so that delay is inside the growing time.



2. Numerical fitting

2.1. Units conversion. Going from fluorescense units to concentration units.

2.2. Fitting the estacionaria phase.

2.3. Fitting the exponential growth.

Referencias

[1] MH Zwietering, Il Jongenburger, FM Rombouts, and K Van't Riet. Modeling of the bacterial growth curve. *Applied and environmental microbiology*, 56(6):1875–1881, 1990.